Mol Gen Genet (1995) 247:399–408

ORIGINAL PAPER

Alfred M. Handler · Sheilachu P. Gomez

The *hobo* transposable element has transposase-dependent and -independent excision activity in drosophilid species

Received: 8 August 1994 / Accepted: 3 October 1994

Abstract Mobility of the *hobo* transposable element was determined for several strains of *Drosophila melanogaster* and several Drosophila species. Mobility was assessed by use of an in vivo transient assay in the soma of developing embryos, which monitored *hobo* excision from injected indicator plasmids. Excision was detected in a *D. melanogaster* strain (*cn*; ry^{42}) devoid of endogenous *hobo* elements only after co-injection of a helper plasmid containing functional *hobo transposase* under either heat shock or normal promoter regulation. Excision was also detected in *D. melanogaster* without helper in strains known to contain genomic copies of *hobo*. In Drosophila species confirmed not to contain *hobo*, *hobo* excision occurred at significant rates both in the presence and absence of co-injected helper plasmid. In four of the seven species tested, excision frequencies were two- to fivefold lower in the presence of plasmid-borne *hobo*. *hobo* excision donor sites were sequenced in indicator plasmids extracted from *D. melanogaster cn*; ry^{42} and *D. virilis* embryos. In the presence of *hobo* transposase, the predominant excision sites were identical in both species, having breakpoints at the *hobo* termini with an inverted duplication of proximal insertion site DNA. However, in the absence of *hobo* transposase in *D. virilis*, excision breakpoints were apparently random and occurred distal to the *hobo* termini. The data indicate that *hobo* is capable of functioning in the soma during embryogenesis, and that its mobility is unrestricted in drosophilids. Furthermore, drosophilids not containing *hobo* are able to mobilize *hobo*, presumably by a *hobo*-related cross-mobilizing system. The cross-mobilizing system in *D. virilis* is not functionally identical to *hobo* with respect to excision sequence specificity.

Key words *hobo* element · Transposon excision
Transposable elements · *Drosophila melanogaster*
Drosophilidae

Communicated by D. J. Finnegan

A. M. Handler (✉) · S. P. Gomez
Insect Attractants, Behavior and Basic Biology Research Laboratory, Agricultural Research Service,
U.S. Department of Agriculture, 1700 S.W. 23rd Dr., Gainesville, Florida 32608, USA

Introduction

Transposable elements are of considerable interest due to their contribution to genetic diversity and genome evolution, as well as their use as genetic tools (see Berg and Howe, 1989). An understanding of a transposable element's function in the cellular environment in which it is usually found, as well as its ability to function in foreign environments, is important to both these considerations. The *Drosophila melanogaster hobo* transposon is of specific interest since nucleotide and amino acid sequence comparisons indicate that it is a member of a broad-ranging family of transposons including those found in plants (Streck et al. 1986; Calvi et al. 1991; Feldmar and Kunze 1991), yet it is one of the most narrowly distributed transposons in *Drosophila* (Daniels et al. 1990). Elucidation of the regulation of *hobo* transposition in drosophilids is important for understanding its phylogenetic distribution, as well as its potential use as a gene-transfer vector in more distantly related insects (Handler and O'Brochta 1991).

hobo is a member of the short terminal inverted repeat class of mobile genetic elements, and was discovered originally in *D. melanogaster* by its association with the *Sgs4* gene (McGinnis et al. 1983; Streck et al. 1986) and a functional full-length 3.0 kb allele was subsequently cloned from the dpp^{dblk} strain (Blackman et al. 1987; 1989; see Blackman and Gelbart 1989 for a review of *hobo*). Like the *P* and *mariner* elements, *hobo* mobility relies on an internally encoded transposase to promote the transposition of its terminal sequences, which has allowed it to be developed into

a bipartite vector-helper transformation system in *D. melanogaster* (Blackman et al. 1989). Beyond their general structural similarities, however, there is no apparent DNA or amino acid sequence homology among these elements nor do they act to mobilize one another. Evidence to date also indicates variability among the transposons in the regulation of their mobility in terms of tissue specificity, as well as strain-specific regulation and phylogenetic restrictions.

Tissue specificity is most clearly defined for *P*, whose movement is restricted to the germline due to tissue-specific splicing of the transposase transcript (Laski et al. 1986). In contrast, the *mariner* element is highly active in the soma as evidenced by significant mosaicism of *mariner*-associated mutant alleles (Haymer and Marsh 1986). For *hobo*, recent genetic evidence indicates that, similarly to *P*, transposase activity is limited to the germline, but unlike *P*, this is most likely to be due to transcriptional regulation and not to tissue-specific intron splicing (Calvi and Gelbart 1994). Although less conclusive, some evidence does exist for the somatic movement of *hobo* based upon somatic polymorphisms in *hobo*-associated mutant phenotypes (Blackman and Gelbart 1989) and chromosomal distribution (Lim 1981; Yannopoulos et al. 1987; Kim and Belayaeva 1991). In this report we consider the potential of *hobo* to function in the soma during early embryogenesis.

Variability also exists among the terminal inverted repeat elements in terms of their distribution among other drosophilids and organisms. While the *P* element (Lansman et al. 1985; Daniels et al. 1990; Anxolébèhere and Periquet 1987) and *P* mobility (O'Brochta and Handler 1988; Handler et al. 1993) is largely restricted to *Drosophila* species, and it is not an apparent member of a larger related family, *mariner* and related elements are found throughout the Insecta (Robertson 1993), and in nematodes as well (Sulston et al. 1992). In contrast, *hobo* appears, thus far, to be the most narrowly distributed transposon in *Drosophila*. Based on DNA hybridization, *hobo* was found to be restricted to several species in the *melanogaster* and *montium* subgroups of the *melanogaster* group (Daniels et al. 1990), and sequence conservation suggests that horizontal transfer of *hobo* may have occurred among at least some of them (Simmons 1992). Nevertheless, a molecular analysis and comparison with other transposons revealed domains of homology in amino acid sequence between *hobo* and the plant transposons *Activator* (*Ac*) from maize, and *Tam3* from *Antirrhinum majus* (Calvi et al. 1991). *hobo* has also exhibited mobility properties in *Musca domestica* and a related element exists in this species (Atkinson et al. 1993; O'Brochta et al. 1994). It is thus somewhat enigmatic that *hobo* is capable of horizontal transmission and has an apparent evolutionary relationship to distantly related transposons, yet is so narrowly distributed in *Drosophila*. We begin to consider this question by determining the range, and to a limited extent, the type of *hobo* function among Drosophilidae. We find that *hobo* mobility is generally unrestricted among drosophilids, and that systems able to cross-mobilize *hobo* apparently exist in all the species tested. However, we find that at least one of the cross-mobilizable systems functions differently from *hobo*, and that several of them may interact negatively with *hobo* to inhibit its mobility.

Materials and methods

Drosophila species and strains

D. melanogaster strains included the E strains devoid of *hobo*, *cn*; ry^{42} and Canton-S, and the H strains which harbor *hobo*, Oregon-R, dpp^{dblk}, and *Bc Elp/CyO*, P[ry^{+}, HBL1]; *ry* (referred to as *hobbled* or *hbl*). Oregon-R contains 10–50 copies of *hobo* (Streck et al. 1986), dpp^{dblk} contains approximately four functional alleles of *hobo* (Blackman et al. 1987), and *hbl* is a transformant strain containing a single *P*-mediated integration of the functional HFL1 *hobo* element having the 3′ terminus deleted (Calvi et al. 1991). The *Drosophila* species *D. melanica*, *D. repleta*, *D. saltans*, *D. simulans*, *D. virilis*, *D. willistoni* and *Chymomyza procnemis* were obtained from the Bowling Green collection. Of these only *D. simulans* is known to contain H strains (Streck et al. 1986). The presence or absence of *hobo* in all experimental strains was confirmed by Southern analysis and polymerase chain reaction (PCR) gene amplification (see Results).

Plasmids

pHFL1

This plasmid contains the 2959 bp *hobo* element from the 94E polytene interval including 49 bp and 405 bp of chromosomal DNA from that region, adjacent to the 5′ and 3′ termini, respectively, inserted into the *Kpn*I-*Sst*I site of pBS-KS (Blackman et al. 1989; Calvi et al. 1991). It was used as a source of *hobo* transposase (helper plasmid) and the basis for other plasmid constructs.

pK19

This plasmid is homologous to pUC19 except that the ampicillin marker has been replaced with a kanamycin resistance gene (Pridmore 1987). It was used as a vector plasmid for pKHFLlacZ, and for control excision experiments in *D. virilis*

pKHFLlacZ

This was used as a *hobo* excision indicator plasmid to assess *hobo* mobility in insect embryos as a result of *hobo* excision deleting the *lacZα* peptide reporter gene. The entire *hobo* element and flanking chromosomal DNA within the *Kpn*I-*Sst*I fragment of pHFL1 was inserted into the pK19 (Pridmore 1987) cloning site. The *lacZα* peptide gene within the *Nde*I-*Afl*III fragment of pUC19 was subsequently inserted into the pHFL1 *Nde*I-*Sca*I sites. The *Nde*I-*Sca*I deletion removes 381 bp within the *hobo* reading frame making it a non-autonomous element. Plasmid construction and analysis followed standard cloning procedures (see Sambrook et al. 1989).

P[ry⁺, HSH2]

This plasmid, referred to as HSH2, has the entire *hobo* transposase open reading frame from pHFL1 under *hsp70* promoter regulation, inserted into a *P* element vector (Calvi et al. 1991; Calvi and Gelbart 1994). It was used as a heat shock-regulated source of *hobo* transposase.

hobo excision assay and product analysis

The *hobo* excision assay was similar in concept and methodology to a *P* element excision assay described previously (O'Brochta and Handler 1988; O'Brochta et al. 1991), and involves the transient expression of plasmid-encoded genes injected into insect embryos allowing the excision activity of the *hobo* element to be assayed (Fig. 1). The pKHFLlacZ kanamycin-resistant indicator plasmid was either injected alone or co-injected into embryos with transposase-encoding helper plasmid. Ampicillin-resistant *hobo* helper plasmids were either HSH2 having the transposase gene under heat shock regulation, or pHFL1 containing an unmodified *hobo*. Embryos were injected under halocarbon oil with an air-pulse injection system using concentrations of 0.3 : 1.0 mg/ml helper : indicator, or 1.0 mg/ml indicator alone, in injection buffer (5 mM KCl, 0.1 mM sodium phosphate pH 6.8). Injected embryos were then incubated in a 100% humidity, oxygenated environmental chamber. After 16–20 h incubation and a 45 min heat shock (37° C), plasmids were harvested from surviving embryos and transformed into bacteria by electrophoration (Bio-Rad Gene Pulser). Optimal plasmid concentrations injected and time of incubation were empirically determined. Transformed bacteria were plated on 5-bromo-4-chloro-3-indolyl β-D-galactopyranoside (X-gal; 40 μg/ml), kanamycin (50 μg/ml) LB media, allowing only indicator plasmid-transformed bacteria to survive. Both precise and imprecise *hobo* excisions deleting the *lacZα* peptide gene resulted in lack of bacterial β-galactosidase activity, yielding white colonies, while blue colonies retain the non-excised plasmid. Excisions or deletions of *hobo* that did not include the internal *lacZ* gene are not detected by this assay. All putative excision plasmids were verified by a single site *Bgl*II restriction analysis and were sequenced in some experiments. Excision frequencies were computed by dividing the number of verified excision events by the total colonies. Total excision frequencies usually resulted from two to five independent injection-bacterial transformation experiments with at least 4×10^4 indicator plasmids assayed.

To control for possible excision or deletion during bacterial transformation, individual plasmids and helper-indicator mixtures were transformed into bacteria, with no subsequent detection of excision (white LacZ⁻ bacterial colonies). Helper plasmids which might have escaped the kanamycin screen, yielding white colonies, were eliminated after restriction digest analysis on the basis of size (> 6 kb).

Sequencing was performed by dideoxy reactions using Sequenase (US Biochemicals), or with the Sequenase Dye Terminator kit (Applied Biosystems) run on an Applied Biosystems 373A automated DNA sequencer. Sequence analysis of excision plasmids was done by alignment with the pKHFLlacZ indicator plasmid using the GeneWorks software (Intelligenetics).

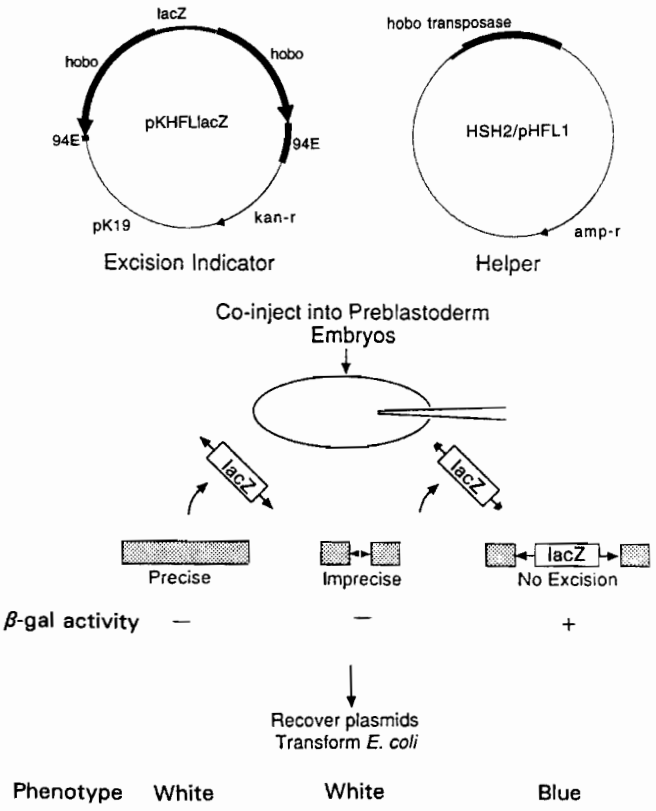

Fig. 1 The transient in vivo *hobo* excision assay using the pKHFLlacZ indicator plasmid including the *lacZα* peptide reporter gene inserted into the *hobo* open reading frame. *hobo* transposase helper is provided by a heat shock promoted (HSH2) or unmodified (pHFL1) *hobo* gene within a separate helper plasmid, or by chromosomal copies of *hobo* within the host insect genome

DNA amplification

Putative *hobo* sequences were amplified from genomic DNA using the polymerase chain reaction in 15–30 μl reactions containing *Taq* DNA polymerace (Boehringer-Mannheim) at 2.5 U/100 μl buffer (10 mM TRIS-Cl, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1 mg/ml gelatin, pH 8.3), with 200 μM dNTPs, 5% glycerol, and 400 nM of each primer. Oligonucleotide primers were non-degenerate sequences identical to the terminal 22 nucleotides at the 5′ and 3′ ends of the *hobo* element in pHFL1 (5′ terminus primer: 5′ CAG AGA ACT GCA AGG GTG GCA T 3′; 3′ terminus primer: 5′ CAG AGA ACT GCA GCC CGC CAC T 3′). Cycling parameters were initial denaturation at 94° C for 1 min, followed by 35 cycles of 1 min at 93° C for denaturation, 1 min at 50° C for annealing, and 2 min at 72° C for extension, with a final extension for 10 min. Amplified products were separated by 1% agarose gel electrophoresis and were stained with ethidium bromide. DNA was transformed to nylon membranes, immobilized by UV-irradiation and hybridized to the ^{32}P-labelled 2.6 kb *hobo Xho*I fragment under moderate stringency conditions.

Results

Somatic function of *hobo*

The somatic activity of plasmid-borne *hobo* transposase was assayed by its ability to promote *hobo* excision from plasmids in the soma of injected embryos. The primary control for this test was the frequency of *hobo* excision in *cn*; ry^{42} E strain embryos not injected with *hobo* helper. Table 1 shows that in *cn*; ry^{42} embryos injected with pKHFLlacZ indicator plasmid alone, *hobo* excision was not observed after scoring more than 72 000 indicator plasmids. On co-injection of HSH2 helper plasmid encoding heat shock-regulated

Table 1 *hobo* excision frequencies in *Drosophila melanogaster* strains

Host strain	Plasmids injected[a]	Number of experiments	pKHFLlacZ screened	Excisions (*n*)	Frequency ($\times 10^{-3}$)
Drosophila melanogaster					
cn; ry^{42}	I	5	72100	0	0
	I + HSH2	8	82240	67	0.81
	I + HFL1	3	40720	18	0.44
hobbled	I	6	47400	18	0.38
	I + HSH2	4	55920	19	0.34
Oregon-R	I	4	40200	34	0.84
	I + HSH2	5	49150	49	1.00

[a] I = pKHFLlacZ indicator plasmid

transposase, an excision frequency of 0.81×10^{-3}/indicator plasmid resulted, while injection of the pHFL1 helper encoding an unmodified transposase gene resulted in an excision frequency of 0.44×10^{-3}. The results indicate that in *cn*; ry^{42}, exogenous *hobo* transposase is required to promote *hobo* excision. Furthermore, *hobo* under normal promoter regulation could promote *hobo* mobility in the embryonic soma, albeit at a reduced level compared to the *hsp70*-regulated *hobo*.

The somatic function of plasmid-borne *hobo* may not reflect the normal in vivo activity of *hobo* in the soma, due to the large number of pHFL1 plasmids injected, which might supersede negative regulatory activity. To determine if *hobo* excision could be catalyzed at detectable levels in the presence of a normal cellular complement of *hobo* genes, excision assays were performed in the *hbl* strain containing a single *P*-mediated *hobo* integration (Calvi et al. 1991; Calvi and Gelbart 1994), and a wild-type Oregon-R strain containing 10 to 50 copies of *hobo* (Streck et al. 1986) per haploid genome. Within both strains excision occurred at nearly equivalent rates in the absence or presence of HSH2 helper (Table 1). Significantly, in Oregon-R, excision occurred at rates comparable to *cn*; ry^{42} with helper, but in *hbl*, excision occurred at an approximately 50% lower rate.

The possibility that some excisions may occur in germline cells was tested directly in previous assays for *P* element excision (Handler et al. 1993) that compared a somatically active *P* helper (phs$\pi\Delta$2-3) to a helper (phsπ) producing functional transposase only in the germline. Tests in *D. melanogaster* showed that phs$\pi\Delta$2-3 promoted excision at a rate of 1.33×10^{-3}/indicator plasmid while phsπ did not promote any observable excision events, indicating that germline excisions are not typically detected by the embryonic excision assays.

hobo presence in drosophilids

Since resident genomic *hobo* elements were able to support significant levels of *hobo* excision in *D. melanogaster*, prior to testing *hobo* mobility in drosophilid species it was necessary to determine whether *hobo* exists in these species. None of these species, except for *D. simulans*, is thought to harbor *hobo* elements based on moderate stringency DNA hybridization studies (Daniels et al. 1990). However, it remains possible that elements could have been missed due to low copy number, large internal deletions, or nucleotide changes eliminating restriction sites. To test further for the presence of *hobo* in these species, and confirm the presence or absence of *hobo* in our *D. melanogaster* strains, we first repeated the Southern analysis (data not shown) which confirmed the results of Daniels et al. (1990). As a more sensitive test we performed PCR using the terminal sequences as priming sites and identified amplified *hobo* sequences by hybridization to the large *hobo* 2.6 kb *Xho*I fragment internal to the priming sites. Although this technique is highly sensitive, it requires conservation of the *hobo* terminal sequences. Figure 2a shows a single 2.95 kb ethidium bromide-stained product from the amplified pHFL1 plasmid control and several products of varying size amplified from the genomic DNA of the various *D. melanogaster* strains and drosophilid species. A strong hybridization signal to the PCR products was deleted in pHFL1, Oregon-R, dpp^{dblk}, and *D. simulans*, all of which are known to contain *hobo* (Fig. 2b). While some hybridization below 3.0 kb may be due to internally deleted *hobo* elements, the strong signal throughout the pHFL1 lane suggests hybridization to incomplete PCR products. After extended autoradiographic exposure, the *cn*; ry^{42} E strain and Canton-S, which purportedly do not contain *hobo*, exhibited no hybridization. The *hbl* strain, which contains only the 5′ terminal sequence primer site also showed a lack of hybridization. Consistent with Southern hybridization studies (Daniels et al. 1990), and despite the presence of stained PCR products, the *D. melanogaster hobo* element was not detected in any of the other drosophilid species. Although the stained products in these species are probably due to random priming events, it is possible that they represent *hobo*-related elements having conserved termini (Streck et al. 1986).

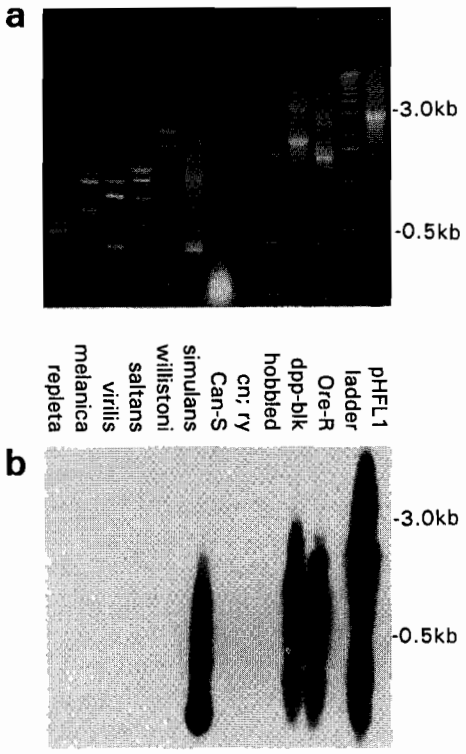

Fig. 2a, b PCR amplification of a pHFL1 plasmid and genomic DNA from *Drosophila melanogaster* strains and *Drosophila* species using primers for the *hobo* terminal sequences. **a** PCR products separated by agarose gel electrophoresis and stained with ethidium bromide. **b** The PCR products in **a** after blotting, hybridization to ^{32}P-labelled *hobo* probe, and extended autoradiography exposure indicating the presence or absence of *hobo*

hobo excision in drosophilids

To determine the limits of *hobo* function within the *Drosophila* genus, the *hobo* excision assay was performed in several drosophilid species, including the distantly related *D. melanica*, *D. repleta*, *D. virilis*, and *C. procnemis* species, and the more closely related *D. simulans*, *D. saltans*, and *D. willistoni* species. Table 2 shows that, with some variability, all the species tested could support *hobo* excision both with and without the co-injection of HSH2 helper plasmid, similar to the results from *D. melanogaster* strains known to contain functional *hobo*. Of these species, a significantly lower level of excision without helper was observed only in *D. saltans*, where excision was nearly sevenfold less. Interestingly, excision frequencies were 2.5- to 5-fold lower with helper in *D. melanica*, *D. repleta*, *D. simulans* and *C. procnemis*. To confirm that *hobo* excision or deletion in the absence of helper is *hobo*-dependent, the pK19 vector host plasmid (for pKHFLlacZ construction) was injected alone into *D. virilis*. The assay of more than 112 000 plasmids revealed that excision or deletion of *lacZ* from pK19 occurred more than 20-fold less frequently compared to pKHFLlacZ injected alone (Table 2), indicating the involvement of the HFL1 sequences in excision or deletion from the indicator plasmid.

Excision site analysis

Excision donor sites from *D. melanogaster cn*; *ry*^{42} and *D. virilis* were sequenced using the pK19 priming sites

Table 2 *hobo* excision frequencies in drosophilid species

Host strain	Plasmids injected[a]	Number of experiments	pKHFLlacZ screened	Excisions (*n*)	Frequency ($\times 10^{-3}$)
Drosophilids					
D. melanica	I	3	35 360	78	2.21
	I + HSH2	5	43 160	45	1.04
D. repleta	I	1	5 940	40	6.74
	I + HSH2	2	20 710	21	1.01
D. saltans	I	1	60 000	7	0.12
	I + HSH2	2	44 100	36	0.82
D. simulans	I	3	64 130	64	1.00
	I + HSH2	3	82 430	39	0.47
D. virilis	I	5	45 800	26	0.57
	I + HSH2	4	46 250	29	0.63
	pK19	2	112 720	3	0.03
D. willistoni	I	3	21 100	91	4.31
	I + HSH2	3	4 600	31	6.74
Chymomyza procnemis	I	4	49 100	66	1.34
	I + HSH2	2	63 840	22	0.34

[a] I = pKHFLlacZ indicator plasmid

94E target site	*hobo*	94E target site	
CGCAGCATCAGGAT ◆⇐	CAGAGA----TCTCTG ⇒◆	ATTCAGGATATCGT	
***D. melanogaster cn; ry*^{42}**			
			n
CGCAGCATCAGGA- ◆	**ATCCTG**	◆ ATTCAGGATATCGT	18
CGCAGCATCAGGA- ◆	**ATCTG**	◆ ATTCAGGATATCGT	3
CGCAGCATCAGGAT◆	**CCATG**	◆ ATTCAGGATATCGT	1
CGCAGCATCAGGA- ◆	**ATCGACATG**	◆ ATTCAGGATATCGT	1
CGCAGCATCAGGA- ◆	**ATCCTG ACTATAT**	◆ ATTCAGGATATCGT	1
◀**GTATAG**▶TCAGGA- ◆	**ATCCTG**	◆ ATTCAGGATATCGT	1
◀ **CTATAT**▶---------- ◆	**ATCCTG**	◆ ATTCAGGATATCGT	1
D. virilis			
CGCAGCATCAGGA- ◆	**ATCCTG**	◆ ATTCAGGATATCGT	9
CGCAGCATCAGGA- ◆	**ATCTG**	◆ ATTCAGGATATCGT	4
CGCAGCATCAGGAT◆	**CCTG**	◆ ATTCAGGATATCGT	1

Fig. 3 Sequence analysis of nearly precise excision donor sites in pKHFLlacZ indicator plasmids after co-injection with HSH2 helper into *D. melanogaster cn; ry*^{42} and *D. virilis*. The sequence on top represents the pKHFLlacZ *hobo* insertion site showing the *hobo* terminal inverted repeat sequences and adjacent 94E chromosomal target site DNA. Sequences below show the types of target site after *hobo* excision. Boundaries of the *hobo* terminal inverted repeat sequences (⇔), boundaries of the 94E chromosomal insertion site DNA (◆ ◆), *n* = number of excision products, duplicated target site DNA is *underlined*, added excision site DNA (having inverted target site duplication motif) is in *bold*, deleted sequences (- - -), and added DNA of unknown origin is placed within (≪ ... ≫)

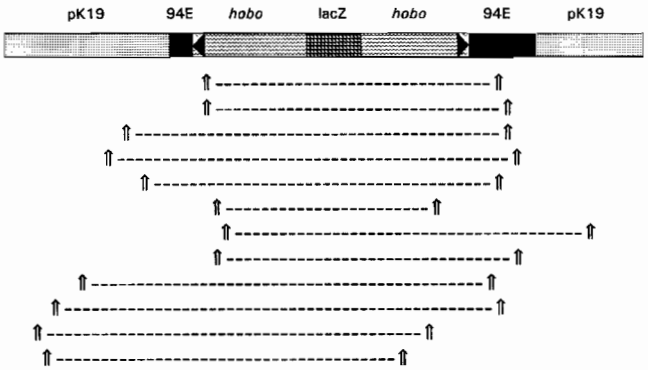

Fig. 4 Breakpoints in the pKHFLlacZ indicator plasmid after imprecise excision or deletion of *hobo* and adjacent DNA after injection into *D. virilis* without helper plasmid. Each pair of *arrows* indicates the approximate breakpoint positions, with the *broken line* indicating the deleted sequence of individual excision plasmids (diagram not to scale)

in pKHFLlacZ proximal to the 94E chromosomal DNA. For excisions recovered in the presence of *hobo* helper in both species, the most frequent excision site sequence was a nearly precise excision of *hobo* with a deletion of the thymidine adjacent to the 5′ terminus and an addition of a 6 bp inverted duplication (ATCCTG) of the original chromosomal integration site (Fig. 3). The second most frequent excision product in both species had a 5 bp inverted duplication (ATCTG). Several other discrete nearly precise excisions in *cn; ry*^{42}, and one in *D. virilis*, had varying amounts of added or deleted adjacent DNA, some including the inverted duplicated motif and the proximal addition of DNA of unknown origin. In addition to the nearly precise excisions, a small number of imprecise excisions (or deletions) occurred with helper in both species ($n = 4$ in *D. melanogaster*; $n = 5$ in *D. virilis*) having breakpoints either within *hobo*, the adjacent chromosomal DNA, or in some cases the pK19 vector DNA. One excision plasmid from *D. virilis* was rearranged.

Excisions from *D. virilis* which occurred in the absence of co-injected *hobo* helper were also sequenced ($n = 12$). All were similar in general structure to the imprecise excision class found in the presence of *hobo* in that breakpoints occurred at various sites in the indicator plasmid (Fig. 4). None of the breakpoints was within 30 bp of the *hobo* termini, nor was duplicated or additional DNA apparent. Among these excisions were several which could not be sequenced, presumably due to deletion of the pK19 priming sites.

Discussion

The data presented indicate that *hobo* is capable of somatic excision during early development, and that rates of *hobo* excision in distantly related drosophilid species are comparable to those in *D. melanogaster*. Furthermore, these species also contain systems capable of mobilizing *hobo*, but which may negatively interact with *hobo* transposase. These findings are in contrast to those for the *P* element, which is completely devoid of somatic function, whose mobility decreases with phylogenetic distance from *D. melanogaster*, and for which cross-mobilizing systems have not yet been identified. The data, however, do reveal and confirm functional similarities to the *Activator* and *Tam3* transposons, to which *hobo* has been previously related based on structure (Streck et al. 1986; Calvi et al. 1991; Feldmar and Kunze 1991) and function (Atkinson et al. 1993).

hobo is capable of somatic function in early development

Several transposable elements retain function in both the somatic and germline tissue, while others are restricted to germline activity. For *hobo*, Calvi and Gelbart (1994) recently presented compelling genetic evidence indicating that *hobo* activity is also restricted to the germline. In contrast, our data indicate that

somatic function of *hobo* can occur during early development, as indicated by the ability of a normally regulated *hobo* element to promote excision of a non-autonomous element. Importantly, this was demonstrated in the presence of a high copy number of plasmid-borne *hobo* genes, as well as chromosomal *hobo* genes present in single and multiple copies per genome.

Calvi and Gelbart (1994) argue that the lack of *hobo* somatic activity is due to limits on transposase transcription. Since in their tests *hsp70*-promoted *hobo* was somatically active, and genomic position effects did not result in somatic activity, they proposed that germline specificity is most likely due to negative regulation of the 5′ promoter sequence in somatic tissue. Given this possibility, one explanation for our results is that the indicator plasmids used in the excision assay act to titrate the putative repressors. Since the *hobo* promoter sequences exist in pKHFLlacZ, sufficient repressor molecules may have been competitively bound, allowing transcription from the helper genes. Thus, while *hobo* activity may be normally restricted in the soma, our data demonstrate that *hobo* function is not absolutely prohibited and suggest that the restriction can be titrated, giving support to a model of active repression. In this respect, further excision tests with specific sequence additions or deletions in the indicator, or in a third plasmid, might give clues to the repressor binding sites and binding kinetics.

Beyond this interpretation, it is also possible that the presence or activity of relevant repressor factors have varying developmental or strain specificities. Whereas Calvi and Gelbart (1994) monitored somatic activity throughout development, and in one test a low level of somatic function was detected, the excision assay tested *hobo* activity only during the preblastoderm stage to mid-embryogenesis, raising the possibility that repressor is not present in early development, or is not maternally derived. For the *Ac* system, to which *hobo* has been related (Calvi et al. 1991; Feldmar and Kunze 1991), somatic transposition also occurs, but is apparently reduced with developmental time presumably due to negative autoregulation that occurs with increasing *Ac* copy numbers (McClintock 1948).

Strain-specific differences also exist for *hobo* activity, as evidenced by recent studies showing that mobility is variably affected by factors both linked and unlinked to *hobo* in different strains (Ho et al. 1993). Although it is not known if the observed regulation involves tissue specificity, somatic movement of *hobo* was previously inferred for particular strains based on *hobo*-associated chromosomal rearrangements in discrete somatic cell populations (Lim 1981; Yannopoulos et al. 1987; Kim and Belayaeva 1991) and the observation of somatic mosaic expression of a *dpp* allele (Blackman and Gelbart 1989). For the nematode transposon *Tc1*, strain differences are clearly demonstrated by somatic activity in both the Bergerac and N2 strains (Emmons and Yesner 1984), with germline transposition limited to the Bergerac line (Collins et al. 1987).

hobo excision is not restricted in drosophilids and is independent of *hobo* transposase

We confirmed that *hobo* elements do not exist in the *cn*; ry^{42} E strain, and, as a critical control for further tests of mobility, we demonstrated that *hobo* excision cannot occur autonomously in this strain. However, as discussed above, in *cn*; ry^{42} *hobo* excision could be promoted *in trans* by an exogenous source of plasmid-encoded *hobo* transposase. In *D. melanogaster* strains containing genomic copies of *hobo*, *hobo* excision could be detected without additional helper plasmid. The rates of excision were generally in the range of $0.4–1.0 \times 10^{-3}$ excision/indicator plasmid assayed. Similar, if not higher rates of excision were also observed in the seven other drosophilid species tested in the presence of *hobo* helper. This is in contrast to *P*, where excision events were more than tenfold less frequent in the distantly related species *D. virilis* and *C. procnemis*, relative to *D. melanogaster* (O'Brochta and Handler 1988; O'Brochta et al. 1991). The similar rates of *hobo* excision in these species, as well as in the others, indicates that *hobo* mobility is not restricted in distantly related drosophilids.

Rather interestingly, and similar to the results from *hbl*, Oregon-R and *D. simulans* which contain *hobo*, all of the other drosophilid species, which we confirmed not to contain *hobo*, supported *hobo* excision in the absence of helper plasmid. Only in *D. saltans* was excision considerably (sevenfold) lower without helper. This would suggest that either *hobo* mobility is not strictly transposase-dependent, or that cross-mobilizable systems, perhaps *hobo*-related, exist in these species. The former suggestion is unlikely given the complete lack of excision without helper in *cn*; ry^{42}. The latter possibility is more likely since a cross-mobilizing system for *hobo* has already been suggested for *M. domestica* (Atkinson et al. 1993), and we currently have evidence for amplification of *hobo*-related sequences from several of the drosophilid species tested, as well as tephritid species, using internal primers which can amplify *Ac* as well as *hobo* (A.M.H. and S.P.G., unpublished). These sequences (approximately 450 bp) show similarity to, but are clearly distinct from the corresponding internal *hobo* sequence and exist as repeated genomic elements (approximately 10–30 copies per haploid genome). Cross-mobilizing activity is also in contrast to *P*, whose excision activity was almost totally transposase-dependent in at least 13 drosophilid and non-drosophilid species tested (O'Brochta and Handler 1988; O'Brochta et al. 1991; Handler et al. 1993). In *D. melanogaster*, however, *P* (Handler et al. 1993) and *hobo* act similarly in that excision only occurred

without helper plasmid in strains having an endogeneous chromosomal source of transposase.

hobo transposase negatively influences excision

In addition to excision occurring at significant levels without helper, in four of the seven *Drosophila* species tested, excision was two- to fourfold higher without helper than with helper. It is difficult to assess definitively the relevance of this result, though the degree and consistency of these data would suggest an ill-defined negative regulatory interaction between *hobo* and the cross-mobilizing systems (or *hobo* in *D. simulans*). A similar conclusion was reached by Atkinson et al. (1993) who observed a similar interaction in *M. domestica* where excision rates were sixfold lower with *hobo* transposase than without. Although the negative regulation of *hobo* in *D. melanogaster*, as well as interactions causing hybrid dygenesis, are not clearly defined, Ho et al. (1993) observed repression of *hobo* activity in several strains of *D. melanogaster* due to a maternal effect involving factors presumably independent of *hobo*, as well as repression in Oregon-R hybrid strains, possibly involving *hobo* elements. Similarly, the observed inhibition of *hobo* excision in the non-*melanogaster* species could be the result of negative interactions with the mobilizing system or other genomic factors.

It is also realized that repression or a decrease in excision may be due to interactions which do not affect the rate of excision, but reduce the fidelity of excision site preference, causing a loss of scorable plasmids due to deletion of replicative or drug resistance functions. Resolution of the actual regulatory interactions between *hobo* and the postulated *hobo*-related elements awaits isolation of the latter so that systematic tests may be performed, either by the transient expression assays discussed here or by transformation of the related elements into appropriate *D. melanogaster* H strains.

Excision site structure

The predominant excision event in both *D. melanogaster cn*; ry^{42} and distantly related *D. virilis*, in the presence of *hobo* transposase, was a nearly precise deletion of the complete *hobo* element with the addition of a six-nucleotide duplication sequence of the original chromosomal DNA insertion site. Several other excision sites showed the same structure except for variations in the number or type of added nucleotides. This confirms a previous finding in a similar analysis of *D. melanogaster* and *M. domestica* (Atkinson et al. 1993), and supports the conclusion that the mode, and possibly mechanism of *hobo* excision is similar to that of the *Ac* and *Tam3* transposons. Unlike the Atkinson et al. (1993) study, we also found a small number of discrete imprecise excision sites in *D. melanogaster* having apparent breakpoints distal to the insertion site.

In *D. virilis*, most of the excisions in the presence of *hobo* transposase were also nearly precise, having the same two predominant types of excision sites as in *D. melanogaster cn*; ry^{42}. In the absence of *hobo* transposase, however, excision presumably catalyzed by a cross-mobilizable system was consistently imprecise, with breakpoints apparently occurring at random or being undirected. When the pK19 vector plasmid, carrying the *lacZ* reporter but no *hobo* sequences, was injected alone, only a very low level of excision or deletion was detected. This indicates that the imprecise excisions in pKHFLlacZ were dependent on sequences in *hobo* or the chromosomal target site, and were not due to a random cutting of foreign DNA. Similar results were obtained in *M. domestica* in that imprecise excision occurred which was *hobo*-dependent, although in this species imprecise events occurred consistently both in the presence and absence of exogenous *hobo* transposase (Atkinson et al. 1993).

Both our study and that of Atkinson et al. (1993) had similar constraints on the sample size and type of excision products which could be sequenced, limiting the conclusions of the respective analyses. The data taken together, however, would indicate that the *hobo* excision processes catalyzed by *hobo* and the cross-mobilizable systems differ. A likely cause for this would be differing or less specific excision sequence site preferences for the cross-mobilizing system. The observation that *hobo* can supersede the activity of the cross-mobilizable system in *D. virilis*, but not in *M. domestica*, would suggest that *Musca* lacks cofactors required for precise *hobo* excision which exist in the *Drosophila* genus, and perhaps more closely related dipterans. Another possibility is that the cross-mobilizable system, or other factors within *Musca* have a negative effect on *hobo* transposase expression or function.

Implications for horizontal movement

Several recent studies which consider the presence or structure of *hobo* in drosophilid species argue that *hobo* has moved among a limited number of species and strains by horizontal transmission (Daniels et al. 1990; Pascual and Periquet 1991; Simmons 1992). Consideration was not given, at least directly, to whether *hobo* mobility is permissive among these species, and if so, whether the narrow distribution of *hobo* is due to functional restrictions on *hobo* movement in other species. Presuming that excision reflects more general mobility properties, our data indicate that *hobo* mobility is generally permissive, though differences do exist in the frequency, and possibly the mechanism of excision. The apparent cross-mobilization of *hobo* would suggest that incomplete or non-autonomous elements, as well as autonomous elements, could be transmitted, and it is

thus worthwhile to consider why *hobo* is not more widely distributed. One possibility generally considered is a relatively brief presence of *hobo* in drosophilids with a recent horizontal transfer, which is suggested by the small number of nucleotide differences in *hobo* elements, among the species that harbor it (Simmons 1992).

A more speculative possibility for the limited transmission of *hobo* suggested by our data is a negative interaction between *hobo* sequence or transposase and the cross-mobilizing system, or other genomic factors, in the new host species. Although excision was not completely repressed in the presence of *hobo* in the excision assays, *P* mobility, which is normally fully repressed in P strains (Engels 1979), was similarly repressed threefold in *P* excision assays in the *D. melanogaster* P strain Harwich, relative to a permissive M strain (O'Brochta and Handler 1988). We presumed that the large number of injected helper plasmids acted partially to overcome the normal repressed state in Harwich. By analogy, *hobo* may be normally repressed in various non-*melanogaster* drosophilids by *hobo*-related systems. As with the *P* element in P strains, repression of transposase activity could prevent *hobo* transposition into a host genome. Alternatively, the interaction may resemble a type of dysgenesis which promotes chromosome destabilization or other effects which either kill or sterilize the host. *hobo* is clearly associated with chromosomal rearrangements in specific *D. melanogaster* strains and/or chromosomes such as *Uc*, dpp^{dblk}, and *23.5MRF*, with *hobo* and other genomic factors being implicated in both the promotion and repression of this activity (Blackman et al. 1987; Yannopoulos et al. 1987; Ho et al. 1993; Sheen et al. 1993). Thus, in some species the action of *hobo* or interaction with resident systems may result in repression of *hobo* integration, or a type of dysgenesis which does not favour survival of the host offspring, limiting transmission of the element.

Acknowledgements Appreciation is extended to Brian Calvi and William Gelbart for sharing *hobo*-containing plasmids and strains, and to Brian Calvi, David O'Brochta, Stephen Miller, and Kenneth Saville for comments on the manuscript and insightful suggestions. This research was supported by a grant from the USDA-NRI-CSRS.

References

Anxolébèhere D, Periquet G (1987) P-homologous sequences in diptera are not restricted to the Drosophiladae family. Genet Iber 39:211–222

Atkinson PW, Warren WD, O'Brochta DA (1993) The hobo transposable element of *Drosophila* can be cross-mobilized in houseflies and excises like the Ac element of maize. Proc Natl Acad Sci USA 90:9693–9697

Berg DE, Howe MM (1989) Mobile DNA. American Society for Microbiology, Washington, D.C.

Blackman RK, Gelbart WM (1989) The transposable element *hobo* of *Drosophila melanogaster*. In: Berg DE, Howe, MM (eds) Moble DNA. American Society for Microbiology, Washington, D.C., pp 523–525

Blackman RK, Grimaila R, Koehler D, Gelbart WM (1987) Mobilization of *hobo* elements residing within the decapentaplegic gene complex: suggestion of a new hybrid dysgenesis system in *Drosophila melanogaster*. Cell 49:497–505

Blackman RK, Macy M, Koehler D, Grimaila R, Gelbart WM (1989) Identification of a fully functional *hobo* transposable element and its use for germ-line transformation of *Drosophila*. EMBO J 8:211–217

Calvi BR, Gelbart WM (1994) The basis for germline specificity of the *hobo* transposable element in *Drosophila melanogaster*. EMBO J 13:1636–1644

Calvi BR, Hong TJ, Findley SD, Gelbart WM (1991) Evidence for a common evolutionary origin of inverted repeat transposons in Drosophila and plants: hobo, Activator, and Tam3. Cell 66:465–471

Collins J, Saari B, Anderson P (1987) Activation of a transposable element in the germline but not the soma of *Caenorhabditis elegans*. Nature 328:726–728

Daniels SB, Chovnick A, Boussy IA (1990) Distribution of *hobo* transposable elements in the genus *Drosophila*. Mol Biol Evol 7:589–606

Emmons SW, Yesner L (1984) High-frequency excision of transposable element Tc*1* in the nematode Caenorhabditis elegans is limited to somatic cells. Cell 36:599–605

Engels WR (1979) Hybrid dysgenesis in *Drosophila melanogaster*: rules of inheritance of female sterility. Genet Res Camb 33:219–236

Feldmar S, Kunze R (1991) The ORFa protein, the putative transposase of maize transposable element *Ac*, has a basic DNA binding domain. EMBO J 13:4003–4010

Handler AM, O'Brochta DA (1991) Prospects for gene transformation in insects. Annu Rev Entomol 36:159–183

Handler AM, Gomez SP, O'Brochta DA (1993) A functional analysis of the P-element gene-transfer vector in insects. Arch Insect Biochem Physiol 22:373–384

Haymer DS, Marsh JL (1986) Germ line and somatic instability of a *white* mutation in *Drosophila mauritiana* due to a transposable element. Dev Genet 6:281–291

Ho YT, Weber SM, Lim JK (1993) Interacting *hobo* transposons in an inbred strain and interaction regulation in hybrids of *Drosophila melanogaster*. Genetics 134:895–908

Kim AI, Belayaeva ES (1991) Transposition of mobile elements *gypsy*, [*MDG4*], and *hobo* in germ-line and somatic cells of genetically unstable mutator strains of *Drosophila melanogaster*. Mol Gen Genet 229:437–444

Lansman RA, Stacey SN, Grigliatti TA, Brock HW (1985) Sequences homologous to the P mobile element of *Drosophila melanogaster* are widely distributed in the subgenus Sophophora. Nature 318:561–563

Laski FA, Rio DC, Rubin GM (1986) Tissue specificity of *Drosophila* P-element transposition is regulated at the level of mRNA splicing. Cell 44:7–19

Lim JK (1981) Site-specific intrachromosomal rearrangements in *Drosophila melanogaster*: cytogenetic evidence for transposable elements. Cold Spring Harbor Symp Quant Biol 45:553–560

McClintock B (1948) Mutable loci in maize. Carnegie Inst Wash Year Book 47:155–169

McGinnis W, Shermoen AW, Beckendorf SK (1983) A transposable element inserted just 5′ to a *Drosophila* glue protein gene alters gene expression and chromatin structure. Cell 34:75–84

O'Brochta DA, Handler (1988) Mobility of P elements in drosophilids and nondrosophilids. Proc Natl Acad Sci USA 85:6052–6056

O'Brochta DA, Gomez SP, Handler AM (1991) P element excision in *Drosophila melanogaster* and related drosophilids. Mol Gen Genet 225:387–394

O'Brochta DA, Warren WD, Saville KJ, Atkinson PW (1994) Transposition of *Drosophila hobo* elements in non-drosophilid insects. Mol Gen Genet 244:9–14

Pascual L, Periquet G (1991) Distribution of *hobo* transposable elements in natural populations of *Drosophila melanogaster*. Mol Biol Evol 8:282–296

Pridmore RD (1987) New and versatile cloning vectors with kanamycin-resistance marker. Gene 56:309–312

Robertson HR (1993) The *mariner* transposable element is widespread in insects. Nature 362:241–245

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York

Sheen F-m, Lim JK, Simmons MJ (1993) Genetic instability in *Drosophila melanogaster* mediated by *hobo* transposable elements. Genetics 133:315–334

Simmons GM (1992) Horizontal transfer of *hobo* transposable elements within the *Drosophila melanogaster* species complex: evidence from DNA sequencing. Mol Biol Evol 9:1050–1060

Streck RD, MacGaffey JE, Beckendorf SK (1986) The structure of *hobo* transposable elements and their insertion sites. EMBO J 5:3615–3623

Sulston J, Du Z, Thomas K, Wilson R, Hillier L, Staden R, Halloran N, Green P, Thierry-Mieg J, Qui L, Dear S, Coulson A, Craxton M, Durbin R, Berks M, Metzstein M, Hawkins T, Ainscough R, Waterson R (1992) The *C. elegans* genome sequencing project: a beginning. Nature 356:37–41

Yannopoulos G, Stamatis N, Monastriati M, Hatzopoulos P, Louis C (1987) *hobo* is responsible for the induction of hybrid dysgenesis by strains of *Drosophila melanogaster* bearing the male recombination factor 23.5 MRF. Cell 49:487–495